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Investigation on the fungal spoilage of crude drugs (1)**

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絲状菌による生薬の変質に関する研究 (1)

Crude drugs, as other plant materials, are the subject of microbial spoilage under certain conditions and this is a problem for the dealers for a long time. In ordinary storage conditions, the water content of crude drugs is too low to allow the growth of bacteria, then only fungal spoilages will arise. The mycoflora of crude drugs is made up of a wide variety of fungi, especially the common saprophytic genera, such as *Aspergillus*, *Penicillium*, and other air, water, dust, and soil borne fungi are quite universal by its very nature.

The factors which determine the range of the mycoflora will be: (1) the pre-harvested position of the crude drug in the plant; (2) the geographical location, climatic, and environmental conditions where the plant was growing; and (3) the treatments and storage conditions after harvest.

The experience of the microbial spoilage of foods and similar materials shows that, in a particular situation, the deterioration is actually caused by only a small proportion of the micro-organisms initially present, so that a *specific* type of spoilage arises in the given variety of materials under normal conditions of storage. This guiding principle will be applicable to the fungal deterioration of the crude drugs.

Under practical conditions, the spoilage type of a crude drug is determined by groups of factors: (1) the initial infection of the substrate; (2) factors depending on the properties of the substrate ('intrinsic' factors), i.e., the nutrient value, the biological structures, and the specific effects of some of the ingredients for certain fungi; (3) the conditions of storage ('extrinsic' factors), i.e., the moisture content of the substrate, oxygen availability, and temperature; (4) the properties of the dominant fungi, for whose influence the term 'implicit' factors has been coined, i.e., differences in the rate of development of the fungi, synergism, and antagonism. The way in which these influence the development of the fungal association is the subject of this paper.

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Material and method

All crude drug samples were kindly supplied by Takashimado Pharmacy, Hongo, Tokyo. These were in storage just as ordinary dealers do. For this investigation, three experimental conditions were made:

temperature 25°C. and relative humidity (R.H.)	75.4%
temperature 25°C. and relative humidity	84.3%
and temperature 25°C. and relative humidity	100.0%

R.H. 75.4 and 84.3% were made by putting Petri plates in desiccators containing the saturated salt solutions of KCl and NaCl, respectively. In R.H. 100%, distilled water was used. For each experiment, one 20 cm-desiccator was used and two Petri plates with a thin layer of a sample were placed in it and incubated at 25°C. The observation and isolation schedule was as follow:

R.H. 75.4% series

- 1st. observation after 7 days
- 2nd. observation after 14 days
- 3rd. observation after 21 days
- 4th. observation and isolation after 28 days

R.H. 84.3% series

- 1st. observation after 4 days
- 2nd. observation after 7 days
- 3rd. observation and isolation after 10 days
- 4th. observation after 14 days
- 5th. observation and isolation after 21 days
- 6th. observation (and isolation, if necessary).....after 28 days

R.H. 100% series

- 1st. observation and isolation after 4 days
- 2nd. observation and isolation after 7 days
- 3rd. observation and isolation after 10 days
- 4th. observation and isolation after 14 days
- 5th. observation (and isolation, if necessary).....after 21 days
- 6th. observation (and isolation, if necessary).....after 28 days

In each observation, dominant species, subdominant species, minor species, and very minor species were determined by naked eyes, and portions of these were taken to microscopes and genus identifications, and in some cases species identifications

were done. In the isolation, each fungal species which developing on the sample was transferred to Petri plates containing acidified potato-dextrose-agar (pH 4.0) or 10% NaCl added potato-dextrose-agar depending on the species, taking precaution to prevent contaminations by unobjective fungi. After appropriate growth, *Aspergillus glaucus* group, other *Aspergillus* and *Penicillium* species, and other genera of fungi were transferred to test tubes containing 20% sucrose Czapek's solution agar, and potato dextrose agar, respectively. In many cases, direct transfers of the developing fungi on the samples in the Petri plates to test tubes were done successfully. These test tube cultures were served for species identifications. In some cases, special media were used for certain fungi.

Experimental result

Ôren, coptis subterranean stem of *Coptis japonica* Makino

R. H. 75.4% : In 28 days, *Aspergillus mangini* (Mangin) Thom and Raper was growing very poorly.

R. H. 84.3% ; In about 14 days, *Aspergillus mangini* (Mangin) Thom and Raper began to develop. The growth in 28 days was poor.

R. H. 100% : Until 7 days, only *Aspergillus glaucus* group was growing, but later *Aspergillus wentii* developed rapidly. In 14 days, *Aspergillus wentii* dominated over other fungi, and the development was very good.

Dominant species: *Aspergillus wentii* Wehmer

Subdominant species: *Aspergillus ruber*, *Aspergillus mangini* (Mangin) Thom and Raper

Minor species: *Aspergillus awamori* Nakazawa

Very minor species: *Rhizopus nigricans* Ehrenberg, *Aspergillus niger* van Tieghem, *Alternaria* sp., *Penicillium* sp. (asymmetrica)

Onji Polygala root of *Polygala tenuifolia* Willdenow

R. H. 75.4% : In 28 days, no fungal development was observed.

R. H. 84.3 : In 28 days, no fungal development was observed.

R. H. 100% : Until 7 days *Aspergillus glaucus* group was predominant, but later *Aspergillus sydowi* and *Mucor silvaticus* developed rapidly. 7 days later, *Aspergillus sydowi* showed very good growth.

Dominant species: *Aspergillus sydowi* (Bain. and Sart.) Thom and Church

Subdominant species: *Aspergillus ruber*, *Aspergillus amstelodami*, *Aspergillus restrictus*, *Mucor silvaticus* Hagen

Minor species: *Aspergillus niger* van Tieghem, *Penicillium frequentas* Westling *Aspergillus ochraceus* Wilhelm

Chimo, Anemarrhena subterranean stem of *Anemarrhena asphodeloides* Bunge
R. H. 75.4%: In about 14 days, *Aspergillus umbosus* Bain. and Sart. began to develop. In 28 days, it showed fairly good growth.

R. H. 84.3%: In 6 days, the growth of *Aspergillus glaucus* group became visible. 5 days later, its development was fairly good.

Dominant species *Aspergillus ruber*, *Aspergillus umbrosus* Bain. and Sart.

R. H. 100%: In the early stage, *Aspergillus glaucus* group was predominant. In 14 days, *Aspergillus glaucus* group and *Penicillium wortmanii* were about equal in their development. 14 days later, *Penicillium wortmanii* dominated and showed very good growth.

Dominant species: *Penicillium wortmanii* Klöcker

Subdominant species *Aspergillus ruber*, *Aspergillus umbrosus* Bain. and Sart.

Minor species: *Aspergillus wentii* Wehmer, *Penicillium implicatum* Biourge, *Aspergillus niger* van Tieghem, Unidentified Dematiaceae (*Fumicola grisea*?)

Very minor species: *Aspergillus ochraceus* Wilhelm, *Rhizopus nigricans* Ehrenberg

Ögi, Astragalus root of *Astragalus Hoantchy* Franchet or related species

R. H. 75.4%: In 28 days, no fungal development was observed.

R. H. 84.3%: In 4 days, *Torula saccharii* (Syn. *Catenularia fuliginea* Saito) became visible. 3 days later, *Aspergillus ruber* appeared. The fungal growth in 28 days was poor and inconspicuous.

Dominant species: *Torula saccharii*

Very minor species *Aspergillus ruber*

R. H. 100%: At the early stage, *Rhizopus nigricans* and *Torula saccharii* were predominantly growing. In 7 days, *Torula saccharii* was inconspicuous according with the development of *Rhizopus nigricans* and *Scopulariopsis* species. In 14 days, *Scopulariopsis* species predominated and its growth was very good.

Dominant species: *Scopulariopsis breviculis* (Sacc.) Bainier, *Scopulariopsis breviculis* var. *glabrum* Thom, *Rhizopus nigricans* Ehrenberg

Minor species: *Aspergillus ruber*, *Aspergillus restrictus*, *Penicillium* sp. (asym-metrica)

Very minor species: *Aspergillus wentii* Wehmer, *Chaetomium* sp. Unidentified fungus (*Fumicola grisea*?), *Trichothecium roseum* Link

Biyakushi, Radix Angelicae root of *Angelica glabra*

- R. H. 75.4% : *Torula saccharii* and *Aspergillus ruber* became visible in 10 and 14 days, respectively. The fungal growth in 28 days was poor.

Dominant species : *Torula saccharii*, *Aspergillus ruber*

- R. H. 84.3% : In 6 days, *Torula saccharii* appeared. Being retarded 1 day, *Aspergillus ruber* became visible. The fungal growth in 28 days was poor.

Dominant species : *Aspergillus ruber*

Minor species : *Torula saccharii*

- R. H. 100% : At the early stage, *Torula saccharii* and *Rhizopus nigricans* predominated. In 7 days, *Torula saccharii* was inconspicuous with the development of *Rhizopus nigricans* and *Aspergillus ruber*. In 14 days, *Scopulariopsis* species appeared and grew rapidly. In 28 days, *Scopulariopsis* species and *Rhizopus nigricans* were predominating, but their growth was relative retarded and not so good comparing to other crude drugs.

Dominant species : *Scopulariopsis brevicaulis* (Sacc.) Bainier, *Scopulariopsis brevicaulis* var. *glabrum* Thom. *Rhizopus nigricans* Ehrenberg

Minor species : *Aspergillus ruber*

Very minor species : *Aspergillus ochraceus* Wilhelm, *Penicillium* sp. (asymmetirca)

Dockatsu, Radix Angelicae polycladae root of *Angelica polyclada*

- R. H. 75.4% : In 7 days *Aspergillus gracilis* began to develop. The growth in 28 days was poor.

Dominant species : *Aspergillus gracilis* Bainier

- R. H. 84.3% : In 3 days, *Aspergillus ruber* began to develop. In 28 days, it showed fairly good growth.

Dominant species : *Aspergillus ruber*

- R. H. 100% : Until 7 days, *Aspergillus glaucus* group was predominating, but in 14 days *Scopulariopsis* species appeared and developed rapidly. The fungal growth in 28 days was very good.

Dominant species : *Scopulariopsis brevicaulis* (Sacc.) Bainier, *Scopulariopsis brevicaulis* var. *glabrum* Thom, *Aspergillus ruber*, *Aspergillus repens*

Minor species : *Aspergillus elegans* Gasperini, *Penicillium* sp. (asymmetrica)

Very minor species : *Aspergillus niger* van Tieghem, *Aspergillus flavus* Link.

Aspergillus wentii Wehmer, *Rhizopus nigricans* Ehrenberg, *Trichothecium roseum* Link

Uikyo, *Foeniculum* seed of *Foeniculum vulgare* Miller

- R. H. 75.4% : In 28 days, the fungal growth was very poor.

Growing species: *Aspergillus ruber*, *Aspergillus mangini* (Mangin) Thom and Raper

- R. H. 84.3% In 7 days, the growth of *Aspergillus glaucus* group became visible. The growth in 28 days was poor.

Dominant species: *Aspergillus ruber*, *Aspergillus mangini* (Mangin) Thom and Raper

Very minor species: *Scopulariopsis brevicaulis* (Sacc.) Bainier

- R. H. 100% : In the early stage, *Aspergillus ruber* was predominant. In middle stage *Aspergillus versicolor* and *Aspergillus ruber* showed good growth. After 14 days *Scopulariopsis* species predominated. The fungal growth in 28 days was very good.

Dominant species: *Scopulariopsis brevicaulis* (Sacc.) Bainier, *Scopulariopsis brevicaulis* var. *glabrum* Thom

Subdominant species: *Aspergillus sydowi* (Bain. and Sart.) Thom and Church.

Aspergillus versicolor (Vuill.) Tiraboschi, *Aspergillus ruber*, *Aspergillus mangini* (Mangin) Thom and Raper.

Minor species: *Chaetomium globosum* Kunze

Very minor species: *Alternaria* sp.

Chikusetsu-ninjin, *Panaxis Rhizoma* subterranean stem of *Panax japonicus* C. A. Meyer

- R. H. 75.4% : In 14 days, a trace of fungal growth was observed. In 28 days the fungal growth was very poor.

Growing species: *Aspergillus ruber*

- R. H. 84.3% : In 7 days, *Aspergillus glaucus* group showed a poor development. The growth in 28 days was relatively good.

Dominant species: *Aspergillus ruber*, *Aspergillus repens*

Minor species: *Penicillium fellutanum* Biourge

- R. H. 100% : Until about 10 days, *Aspergillus glaucus* group was predominant. Later, *Aspergillus versicolor* and *Scopulariopsis brevicaulis* began to develop. In 21 days, *Aspergillus versicolor* and *Scopulariopsis brevicaulis* dominated and their growth was very good.

Dominant species: *Aspergillus versicolor* (Vuill.) Tiraboschi

Subdominant species: *Scopulariopsis brevicaulis* (Sacc.) Bainier

Minor species: *Aspergillus ruber*, *Aspergillus repens*, *Aspergillus umbrosus* Bain. and Sart. *Penicillium frequentans* Westling *Penicillium* sp. (asymmetrica.) *Tricothecium roseum* Link.

Very minor species: *Aspergillus ochraceus* Wilhelm, *Rhizopus nigricans* Ehrenberg, *Scopulariopsis brevicaulis* var. *glabrum* Thom, *Verticillium* sp. (colonies brick red), Unidentified (yellow mycelia)

Kanzo, Glycyrrhiza root and subterranean stem of *Glycyrrhiza uralensis* Fischer et De Candolle and related species

R. H. 75.4% No fungal development was observed in 28 days.

R. H. 84.3%: In 12 days, *Aspergillus mangini* began to appear. The growth in 28 days was relatively good.

Dominant species: *Aspergillus mangini* (Mangin) Thom and Raper

R. H. 100%: In 3 days, *Aspergillus umbrosus* appeared, and predominated until about 10 days. Later, *Penicillium variable* began to develop rapidly and predominated in 14 days. The fungal growth in 20 days was very good.

Dominant species: *Penicillium variable* Sopp

Subdominant species: *Aspergillus umbrosus* Bain. and Sart

Minor species: *Aspergillus niger* van Tieghem

Very minor species: *Aspergillus ochraceus* Wilhelm, *Circinella* sp. *Aspergillus flavus* Link

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